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Effect of Ozone on Serum Lipids and Lipoproteins in the Rat

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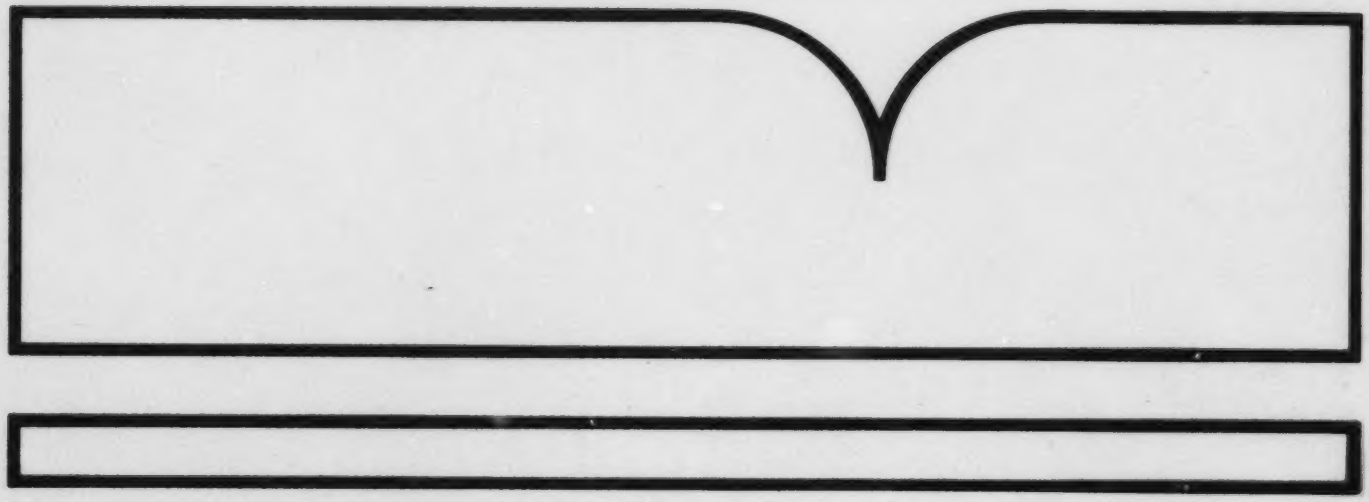

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Effect of Ozone on Serum Lipids and Lipoproteins in the Rat[1]

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Effect of Ozone on Serum Lipids and Lipoproteins in the Rat. MOLE, M. L., JR., STEAD, A. G., GARDNER, D. E., MILLER, F. J., AND GRAHAM, J. A. (1985). *Toxicol. Appl. Pharmacol.* **80**, 367–376. Exposure of male rats to 0 (air), 1, 1.75, and 3 ppm ozone (O_3) 5 hr/day for a total of 10 days resulted in a positive linear relationship between ozone concentration and the concentrations of serum total lipoprotein free cholesterol (FCh) and high-density lipoprotein total cholesterol (HDL-Ch). The latter response was reflected in both its free (HDL-FCh) and esterified (HDL-ChE) components. On the other hand, serum triglycerides (TG) showed a marked decreasing linear trend with increasing ozone concentration. As judged by decreased body weights with no accompanying differences in feed consumption, apparent metabolic rate increased as ozone concentration increased. In another experiment, male rats were exposed 5 hr/day to either air or 1 ppm O_3 for a total of 15 days. Groups of animals from each exposure were sampled at times ranging from immediately after to 44 hr postexposure. In agreement with the concentration response study, effects of O_3 included increases in serum total cholesterol (Ch), HDL-Ch and HDL-FCh, and a decrease in TG. In addition, the degree of effects appeared to be maintained over the 44-hr period and to be greater than that observed at 1 ppm O_3 in the concentration–response study. © 1985 Academic Press, Inc.

Studies of the toxicity of O_3 are numerous and are the subject of reviews (U.S. Environmental Protection Agency, 1978; Natl. Acad. Sci., 1977). Most reports have dealt with effects on the pulmonary system although extrapulmonary effects have been observed. It has been generally assumed that the latter effects, if other than secondary to responses initiated in the lung, are not produced by O_3 itself, but rather by oxidized substances of the lung which enter the systemic circulation and are transported to remote sensitive tissues and organs where primary responses are elicited.

Despite recent declines, cardiovascular diseases still remain the leading causes of death in the United States and, indeed, accounted for over half of all deaths in the U.S. in 1980 (Levy and Moskowitz, 1982). Of the risk factors which have been associated with the predisposition of individuals to cardiovascular diseases, those with the strongest influence are hypercholesterolemia, hypertension, and cigarette smoking (National Heart, Lung, and Blood Institute, 1981). The relationship between air pollution and cardiovascular disease has been the subject of extensive review (Hackney, 1976; Rosenman, 1979; Van Stee, 1982; Shakman, 1974; Topping, 1977), but there are few studies which have addressed

[1] This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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the possibility that O_3 inhalation may contribute to the development of cardiovascular diseases or their associated risk factors. It has been reported that exposure to O_3 induced pulmonary arterial lesions (P'an *et al.*, 1972; P'an and Jegier, 1972) and damage to myocardial fibers (Brinkman *et al.*, 1964) in rabbits, and decreased blood pressure in rats (Drew *et al.*, 1983).

Although the rat species is a poor model in the study of atherosclerosis, it is nevertheless a good model for lipid metabolism studies and as such has been frequently used (Havel, 1980; Brown *et al.*, 1980; Alaupovic, 1981). There have been instances reported of extrapulmonary effects in the rat after O_3 treatment, which may be related to disturbances in lipid metabolism. For example, exposure of young male Sprague–Dawley rats to 1.1 ppm O_3 for 5 to 7 days produced an increase in the activity of serum lecithin:cholesterol acyl transferase (Shimasaki *et al.*, 1976). Also noted in this report was an increase in serum free cholesterol after two 24-hr exposures with a 3 day recovery period in between. Thyroid function in young male Sprague–Dawley rats has been observed to be affected after O_3 inhalation (Fairchild *et al.*, 1964; Clemons and Garcia, 1980). This effect could possibly be further manifested in altered lipid metabolism since it is known that a dysfunction of the thyroid can affect concentrations of circulating lipids, lipoproteins, and the activity of a key regulatory enzyme, lipoprotein lipase (Vanhaelst and Bastenie, 1979).

Given these few but relevant observations, we were encouraged to initiate studies in rats to determine what effects short-term exposure to O_3 would have on serum lipid and lipoprotein profiles. As it has been shown that female and older male rats are less susceptible to effects of O_3 (Fairchild and Graham, 1963; Stokinger and Coffin, 1968) and the rate of cholesterol synthesis decreases with age in rats (Kritchevsky *et al.*, 1980), the young male Sprague–Dawley strain of rat was chosen as the animal model for these studies.

METHODS

Animals. All studies used 70 to 80-day-old male Sprague–Dawley rats (CD-1 strain) obtained from Charles River Farms (Wilmington, Mass.). They were acclimated at least 2 weeks prior to beginning experimentation and were allowed free access to feed and water throughout the studies except during exposure periods. Adequate amounts of stock Rodent Laboratory Chow 5001 (Ralston Purina Company, St. Louis, Mo.) from one milling date was reserved and refrigerated to complete a study. The animals were housed individuallly in wire bottom cages. Body weights and feed intake were recorded twice a week.

Ozone exposure. Animals were exposed to either O_3 or to charcoal and high efficiency particulate aerosol (HEPA) filtered room air while caged individually in stainless steel wire cages inside Rochester-type chambers (Hinners *et al.*, 1968) (Young and Bertke, Cincinnati, Ohio). Ozone was generated from 100% O_2 by an Orec Model 03V5-0 ozonizer (Ozone Research and Equipment Corp., Phoenix, Ariz.), and its concentration was monitored continuously with a chemiluminescence O_3 analyzer (Bendix Model 8003, Bendix Corp., Ronceveite, W.V.). The analyzer was calibrated every 2 weeks by an ultraviolet photometric procedure (Fed. Reg., 1979). Calibration drift during the exposure periods did not exceed 3.2%. Gas flow through all chambers was at the rate of one chamber volume per minute.

Experimental Design

(a) Concentration–response study. The LC50 for rats exposed to O_3 has been reported to be in the range of 3.6 to 6.4 ppm for a 4-hr period (Stokinger, 1962; Henschler, 1973). Therefore, it was felt that 3 ppm should be the highest concentration used in the present experiment. The concentration–response study involved 36 rats which were randomly divided into four groups of nine animals, ear-tagged, and exposed to filtered air (controls), 1960 $\mu g\ O_3/m^3$ (1 ppm), 3430 $\mu g\ O_3/m^3$ (1.75 ppm), or 5880 $\mu g\ O_3/m^3$ (3.0 ppm) for 5 hr/day beginning between 7:30 and 8:30 AM. There were a total of 10 exposure days within a 14-day period according to the following regimen: 2 days of exposure, 2 days no exposure, 5 days of exposure, 2 days no exposure, and 3 days of exposure. Blood samples were taken 2 days prior to the first exposure and approximately 20 hr after the final exposure between 8:00 and 10:00 AM. In both instances, rats were fasted 18 hr prior to drawing 2.5 to 3 ml of blood by cardiac puncture. To perform this procedure, animals were anesthetized with a 5% halothane–air mixture (prepared using a Fluotec 3 vaporizer, Cyprane Ltd., Keighley, England). Blood was drawn in a rotating manner among all groups using one animal per treatment

group in each cycle. No attempt at randomizing the animals for drawing blood was made but the pre- and postsampling orders for individual animals were the same.

(b) Sampling time study. This experiment was conducted to determine if the levels of effects changed over a 2-day time period after the final exposure. In addition, it was of interest to determine the effect on magnitude of responses by increasing the number of exposure days. Since the concentration response study had indicated that some parameters were subject to alteration in response patterns above 1.75 ppm O_3 (Fig. 2), we wished to remain considerably below that but at the same time work at a concentration we felt would elicit a response; therefore, 1 ppm O_3 was chosen for this experiment. The concentration–response study also indicated that based on weight gain, recovery of the animals after drawing blood was not complete until the third day; therefore, for this experiment the first exposure was delayed until the fourth day after blood sampling. A total of 60 animals were divided into 14 groups of 4 or 5 animals each and exposed to air (7 groups) or to 1 ppm O_3 (7 groups) for 5 hr/day beginning between 7:00 and 8:00 AM. There were a total of 15 exposure days in 5-day segments within a 19-day period. Blood was drawn from fasted animals as described for the concentration response study 4 days prior to the first exposure and 0, 4, 9, 14, 20, 32, and 44 hr after the final exposure. Blood sampling times were at 1 PM, 5 PM, 10 PM, 3 AM, 9 AM, 9 PM, and 9 AM, respectively. Due to the complexity in logistics associated with fasting, exposing, and drawing blood, it was necessary to have two fasting periods for the 14-, 20-, 32-, and 44-hr groups. In addition, the fasting time range among the groups just prior to drawing blood was 10 to 23 hr.

Lipid and lipoprotein analyses. Serum was separated by centrifugation after allowing the blood to clot at ice-bath temperature. Aliquots of 200 μl of serum were sampled, and both VLDL and LDL were precipitated from HDL according to a procedure for human sera (National Heart and Lung Institute, 1974) except that precipitation was allowed 1 hr and the samples were then centrifuged at 12,000*g*. Aliquots of 150 μl of whole and precipitated sera were extracted for lipid analyses by a modification of the Bligh and Dyer method (Bligh and Dyer, 1959; Hanson and Olley, 1963). After adding the serum to 564 μl of methanol:chloroform (2:1), the resulting solution and precipitate were mixed for 30 sec and allowed to stand 10 min. Chloroform (189 μl) was added, followed by 189 μl of water with a 15-sec mix on a vortex after each addition. A few crystals of sodium chloride were added to the whole serum extracts, prior to the final mixing, to promote good phase separation. The biphasic mixture was then centrifuged for 10 min at 1500*g*, and the bottom layer was stored at −20°C until assayed for lipids.

Quantitation of the neutral lipids, FCh, ChE, TG, and free fatty acids (FFA) was accomplished by a modification of the procedure described by Touchstone *et al.* (1978). Cholesterol and cholesteryl stearate were supplied by Sigma Chemical Company (St. Louis, Mo.); triolein and oleic acid by Supelco, Inc. (Bellefonte, Pa.). Thin-layer chromatographic plates were 20 × 20 cm of 250-μm silica gel G thickness and were purchased from E. Merck, Darmstadt, West Germany. They were scored into 10-mm wide lanes with a scoring device (Schoeffel Instrument Division, Kratos, Inc., Westwood, N.J.), prewashed by ascension in chloroform:methanol (1:1), and allowed to dry for at least 5 min before spotting. Plates were spotted according to the data-pairing technique (Bethke *et al.*, 1974) with 30 μl of standards and samples using an automatic 18-place spotter (Analytical Instrument Specialities, Inc., Libertyville, Ill.) at 60°C. Six standards over a 15-fold concentration range were used.

The chromatographic chamber, which contained the mobile phase hexane:diethyl ether:acetic acid (80:20:1) and two blank chromatographic plates facing each other to serve as wicks, was equilibrated at least 30 min prior to use. After chromatography, the spotted plates were air dried and sprayed with a solution of 30 g/liter of cupric acetate in 5.6% phosphoric acid. After allowing time for excess reagent to drain, the lipid components were visualized by heating the plate in an oven, placed against an aluminum block, at approximately 190°C for 15 min. The density of the standard and sample spots were measured by spectrodensitometry (Schoeffel Model 3000 spectrodensitometer and 300D density computer) at 365 nm in the single beam reflectance mode using a 7 mm beam width. Standard curves of peak area as either square root or quadratic functions of the mass were generated by digitization (Model 9864A digitizer, Hewlett–Packard, Palo Alto, Calif.), and sample amounts were determined from the standard regression curves.

Cholesterol (Ch) present in esterified form (ChE) was approximated utilizing the MW ratio of cholesterol to cholesteryl stearate, 0.592. The Ch in any given assayed fraction was then determined by summing the FCh (free cholesterol) and ChE values of that fraction. The Ch in precipitated serum represented that associated with HDL. The ratio of Ch/TG (triglyceride) for rat VLDL is similar to that of human VLDL (Lasser *et al.*, 1973); therefore, LDL-Ch was estimated by the equation of Friedwald *et al.*, (1972): LDL-Ch = (Ch) − (HDL-Ch) − TG/5, where TG/5 represents an estimate of the Ch concentration for VLDL.

Statistical Methods

(a) Concentration–response study. Multivariate analysis of variance (MANOVA) (Morrison, 1976) was used to test simultaneously for overall changes with O_3 concentration in the differences between post- and preexposure

concentrations of the lipid classes across each lipoprotein. Individual contrasts were constructed to test responses to each ozone concentration against the control.

In addition, traditional regression techniques (Draper and Smith, 1966) were employed to test for linear trends in lipid values with increasing O_3 concentration. Since multiple animals were used at each concentration, a lack of fit test was performed to test the adequacy of the linear model assumption.

(b) Sampling time study. MANOVA was used to test for ozone vs control and for sampling time effects on the multidimensional response comprised of differences between post- and prexposure concentrations of the lipid classes across each lipoprotein. Univariate analysis was also used when the aggregate response due to ozone exposure was significant.

The p values < 0.1 are considered significant.

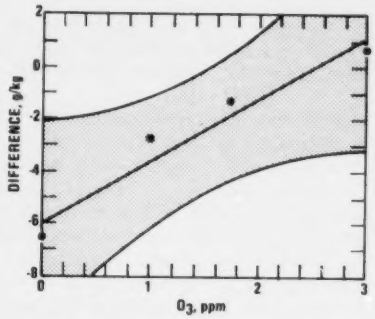

FIG. 1. The response to O_3 concentration of pre- and postexposure differences in the ratio of feed intake to body weight. Shaded area represents 95% confidence limits.

RESULTS

Concentration–Response Study

Other than decreased motor activity during exposure and a smaller rate of increase in body weight, O_3-exposed animals showed no signs of toxicity. Body weights and feed consumption data are presented in Table 1, and a trend is apparent in the rate of change for body weights. The rate of increase in body weight became less while the rate of feed intake was not affected at the higher O_3 concentrations, suggesting an increase in metabolic rate. The differences between pre- and postexposure values for the weight of feed eaten per kilogram of body weight (Fig. 1) produced a significant ($p < 0.04$) linear regression on O_3 concentration.

Concentrations of lipids measured in serum prior to and after O_3 exposure are presented in Table 2. It appeared that some of the differences between group mean concentrations prior to exposure were as large or larger than within group changes due to exposure. Therefore, to evaluate more clearly the effects, each animal was used as its own control and

TABLE 1

EFFECT OF O_3 CONCENTRATION ON RAT BODY WEIGHT AND FEED CONSUMPTION[a]

Group (ppm O_3)	No. of animals	Body weight[b]			Feed consumption[c]		
		Exposure			Exposure		
		Pre	Post	Δ	Pre	Post	Δ
0	9	315 ± 3	355 ± 7	40.4 ± 6.0	27.7 ± 0.5	29.0 ± 1.0	1.28 ± 0.92
1	9	312 ± 7	353 ± 10	41.3 ± 4.0	27.2 ± 0.5	29.8 ± 0.9	2.66 ± 0.58
1.75	8	315 ± 7	349 ± 12	35.1 ± 4.7	27.5 ± 1.3	30.2 ± 0.8	2.74 ± 1.38
3.0	9	314 ± 6	331 ± 8	17.0 ± 3.4[d]	28.1 ± 1.1	29.8 ± 0.7	1.68 ± 0.74

[a] See Methods for exposure regimen.
[b] $\bar{x} \pm$ SE expressed in g.
[c] $\bar{x} \pm$ SE expressed in g/day.
[d] Significantly different from air animals, $p < 0.05$.

TABLE 2

EFFECT OF O_3 CONCENTRATION ON RAT SERUM LIPIDS[a]

Group (ppm O_3)	No. of animals	Total lipoprotein[b]				High-density lipoprotein[b]		
		Cholesterol				Cholesterol		
		Free	Esterified[c]	Triglycerides	Free fatty acids	Free	Esterified[c]	Triglycerides
Preexposure								
0	9	7.19 ± 0.54	60.1 ± 5.1	45.6 ± 8.5	16.3 ± 0.9	4.26 ± 0.44	49.3 ± 3.8	3.61 ± 0.54
1	9	7.87 ± 0.66	61.5 ± 5.0	39.9 ± 8.6	16.8 ± 0.9	4.60 ± 0.47	49.1 ± 3.6	2.18 ± 0.20
1.75	8	6.61 ± 0.41	53.2 ± 2.5	42.2 ± 9.5	15.0 ± 0.4	3.89 ± 0.35	41.9 ± 2.7	2.25 ± 0.37
3	9	7.68 ± 0.60	60.9 ± 3.6	33.9 ± 8.3	15.6 ± 0.6	4.95 ± 0.39	50.3 ± 2.7	2.43 ± 0.46
Postexposure								
0	9	7.22 ± 0.59	62.1 ± 4.0	48.2 ± 7.5	14.7 ± 1.0	3.88 ± 0.48	46.8 ± 4.5	4.21 ± 0.42
1	9	8.15 ± 0.81	64.0 ± 4.2	41.3 ± 4.8	13.0 ± 0.6	4.24 ± 0.33	48.0 ± 3.1	4.56 ± 0.52
1.75	8	7.80 ± 0.53	58.6 ± 3.0	35.2 ± 7.2	13.7 ± 0.5	4.07 ± 0.34	43.9 ± 2.5	2.40 ± 0.52
3	9	9.10 ± 0.56	64.4 ± 2.9	22.1 ± 5.0	12.0 ± 0.9	5.78 ± 0.41	54.7 ± 2.7	3.34 ± 0.46
Pre- and postdifference								
0	9	0.030 ± 0.233	2.08 ± 2.24	2.56 ± 5.77	−1.60 ± 0.62	−0.380 ± 0.270	−2.12 ± 1.77	0.602 ± 0.57
1	9	0.273 ± 0.650	2.49 ± 3.32	1.40 ± 5.27	−3.85 ± 0.92	−0.360 ± 0.360	−1.16 ± 1.74	2.41 ± 0.56
1.75	8	1.19 ± 0.223[d]	5.36 ± 1.21	−6.98 ± 4.84	−1.37 ± 1.10	0.175 ± 0.285	2.03 ± 2.09	0.149 ± 0.50
3	9	1.42 ± 0.333[e]	3.47 ± 2.17	−11.8 ± 3.80	−3.58 ± 0.55	0.824 ± 0.212[e]	4.40 ± 1.84[e]	0.796 ± 0.61

[a] See Methods for exposure regimen.
[b] All data are $\bar{x} \pm$ SE expressed as mg/dl.
[c] Cholesterol as cholesteryl esters approximated as 0.592 × conc. of cholesteryl esters.
[d] Significantly different from air animals, $p < 0.1$.
[e] Significantly different from air animals, $p < 0.05$.

statistical analyses were performed on the resulting differences between post- and preexposure concentrations. Data were not adjusted for pre- and postexposure differences in body weight and dietary intake since statistical analysis showed no significant correlations between these parameters and the differences in the lipids of interest which were not attributable to changing O_3 concentration. Differential lipid concentrations which tended to increase with increasing O_3 concentration were both cholesterol components, FCh and ChE, associated with both the total lipoprotein and HDL. By contrast an inverse relationship existed between differential concentrations of TG and ozone concentration. There appeared to be no effect on HDL-TG or on total lipoprotein free fatty acids. Analysis of variance showed the differential concentration of FCh in total lipoprotein to be significantly greater than control value at 3 ppm O_3 ($p < 0.05$) and marginal at 1.75 ppm O_3 ($p < 0.1$). Also, the differential concentration of FCh and ChE in HDL at 3 ppm O_3 were significantly ($p < 0.05$) elevated over control values.

The effect of O_3 on the lipoprotein lipid profiles is depicted graphically in Fig. 2. The relatively smooth linear changes with increasing O_3 concentration observed for total lipoprotein FCh differences and TG differences, and all HDL cholesterol component differences are contrasted with the apparent discontinuities occurring above 1.75 ppm O_3 for differential total lipoprotein Ch and ChE and for differential LDL-Ch. Simple linear regression analyses of HDL-Ch differences (panel B, Fig. 2) and total lipoprotein TG differences (panel C, Fig. 2) on O_3 concentration were significant ($p = 0.004$ and 0.02, respectively). In the case of total lipoprotein TG, differences were inversely related to the O_3 concentration.

Individual contributions of FCh and ChE to the concentration response relationships of total lipoprotein Ch and HDL-Ch can be seen also in panels A and B of Fig. 2. Significant linear concentration response pat-

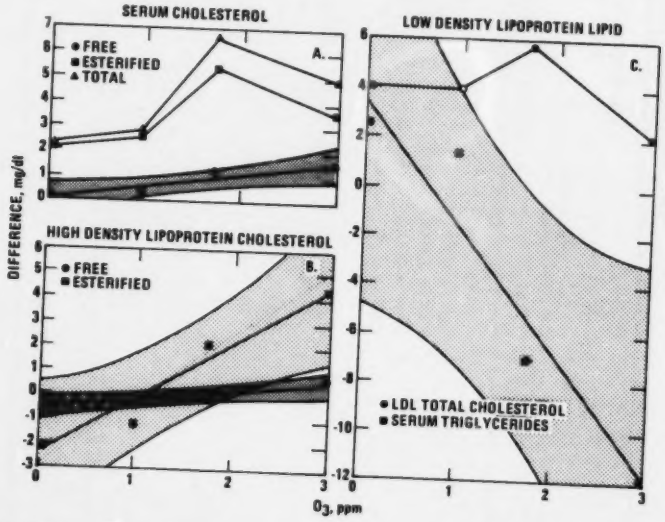

FIG. 2. The responses to O_3 concentration of pre- and postexposure differences in rat serum lipoprotein lipids. Shaded areas represent 95% confidence limits. (A) Ch, FCh, and ChE difference curves for whole serum; (B) FCh and ChE difference curves for serum after precipitation of VLDL and LDL; Ch difference curve is not shown but was very similar to that of ChE. (C) Upper curve represents LDL-Ch differences, lower curve represents TG differences for whole serum.

terns were detected for total lipoprotein FCh differences ($p = 0.009$), HDL-FCh differences ($p = 0.002$), and HDL-ChE differences ($p = 0.008$). Linear concentration response patterns were not evident for total lipoprotein Ch, its esterified component, or LDL-Ch. The discontinuity occurring above 1.75 ppm O_3 observed in the total lipoprotein Ch response can clearly be associated with its esterified component. Apparently a similar discontinuity exists in the LDL-Ch response.

Sampling Time Study

Data resulting from sera taken from rats at various times prior to exposure and following the final exposure to either air or 1 ppm O_3 are presented in Table 3. The data are expressed as the differences in lipid concentrations of serum samples taken from animals before and after their exposure. The MANOVAs indicated significant overall effects due to O_3 exposure on the differential lipid concentrations in both unprecipitated serum (Wilks' $F(4,45) = 2.68$, $p = 0.04$) and precipitated serum (Wilks' $F(4,45) = 3.53$, $p = 0.01$). Individual analysis of variance could not detect an interaction between time of sampling and ozone exposure for any of the responses examined; therefore, the data were pooled across sampling times into an air group and O_3 group to test for the effect of O_3 exposure on individual lipid components. The increase in serum Ch, 3.7 mg/dl, was

TABLE 3

CHANGES IN CONCENTRATIONS OF RAT SERUM LIPIDS AT VARYING TIME PERIODS AFTER EXPOSURE OF THE ANIMALS TO 1 ppm O_3[a]

Hours postexposure	Total lipoprotein[b]			High-density lipoprotein cholesterol[b]	
	Cholesterol				
	Free	Esterified[c]	Triglycerides	Free	Esterified[c]
Air					
0	2.68 ± 1.32	5.40 ± 2.54	27.2 ± 21.2	2.02 ± 0.58	12.2 ± 4.2
4	1.82 ± 1.12	1.05 ± 3.44	−0.15 ± 13.5	3.27 ± 0.25	7.62 ± 2.85
9	5.04 ± 0.68	7.40 ± 3.95	24.3 ± 8.51	1.39 ± 0.38	3.45 ± 5.20
14	4.60 ± 0.81	10.8 ± 2.08	62.6 ± 10.4	0.78 ± 0.57	16.4 ± 7.9
20	4.15 ± 0.83	3.92 ± 1.98	39.8 ± 14.5	−0.18 ± 0.66	10.4 ± 3.6
32	5.41 ± 1.04	5.67 ± 2.69	11.6 ± 6.7	2.95 ± 0.25	7.82 ± 4.11
44	4.24 ± 1.03	5.91 ± 4.55	31.7 ± 22.1	2.00 ± 0.39	2.98 ± 3.24
Mean (Pooled)	4.0 ± 0.4	9.7 ± 2.1	28.1 ± 5.4	1.7 ± 0.2	14.7 ± 2.7
O_3					
0	3.88 ± 1.15	10.0 ± 2.2	5.48 ± 20.5	3.22 ± 0.79	8.38 ± 4.88
4	2.81 ± 0.92	8.58 ± 2.65	−15.0 ± 13.4	4.66 ± 0.88	9.75 ± 5.84
9	5.11 ± 1.38	11.2 ± 2.7	23.4 ± 12.2	2.56 ± 0.39	10.2 ± 2.0
14	5.72 ± 0.26	8.46 ± 4.98	35.8 ± 9.2	0.88 ± 1.44	17.1 ± 1.45
20	6.40 ± 0.89	9.65 ± 1.97	46.0 ± 11.9	1.28 ± 0.81	16.0 ± 2.0
32	4.90 ± 0.96	7.16 ± 3.60	−7.62 ± 4.30	3.25 ± 0.47	9.77 ± 5.75
44	5.30 ± 1.81	4.39 ± 3.50	3.40 ± 5.74	3.58 ± 0.53	10.8 ± 3.8
Mean (Pooled)	4.9 ± 0.4	14.4 ± 2.1	13.1 ± 5.4	2.8 ± 0.2	19.8 ± 2.7

[a] See Methods for exposure regimen.

[b] $\bar{x} \pm$ SE of differences in pre- and postexposure values expressed as mg/dl; all means are based on 4 animals except those in the air group, $N = 3$ at 14 hr and $N = 5$ at 44 hr.

[c] Cholesterol as cholesteryl esters approximated as 0.592 × conc. of cholesteryl esters.

marginal ($p = 0.1$); the serum TG difference was decreased ($p = 0.06$) by 15 mg/dl; the HDL-Ch difference increased ($p = 0.08$) by 4.1 mg/dl; and the HDL-FCh difference increased ($p = 0.006$) by 1.1 mg/dl. The esterified component difference of HDL-Ch showed a response in the same direction as in the concentration–response study; however, the effect was not significant ($p = 0.15$).

Although the direction of effects on all components was consistent for the concentration–response and sampling time studies, the absolute values of the two data sets differed somewhat, particularly for TG and HDL-ChE. The reason for this difference is unknown but may be related to the dissimilar fasting regimens of the two studies. Nevertheless, when one adjusts for these absolute value discrepancies by expressing data for O_3-treated animals as percentages of that for control animals, the magnitudes of effects in the sampling time study are two- to threefold greater than those derived from the concentration–response regression curves at 1 ppm O_3. Since there were 5 additional exposure days in the sampling time study, an increase in the levels of effects might have been anticipated.

DISCUSSION

The studies presented here indicate the following: concentrations of several components of the circulating lipid profile examined in young male rats are affected in a linear concentration-dependent manner up to 3 ppm O_3; effects on the circulating lipid profile are increased and for at least one of the components becomes significant at the lowest concentration tested, 1 ppm 0_3, when the number of exposures are increased from 10 to 15; responses to O_3 exposure among the lipid classes are varied; and based on changes in body weight and feed consumption, metabolic rate is increased in a linear concentration-dependent manner by exposure to ozone. Ozone exposure for 5 hr during the day over a 2- to 3-week period simultaneously induced mild hypercholesterolemia and hypotriglyceridemia. The changes in lipid concentrations caused by 1 ppm O_3 exposure appear to be sustained for at least 2 days after termination of exposure. These results, together with the observation of a greater effect when the duration of O_3 exposure is increased, suggest the responses may be of a cumulative nature. It is possible that the maximum responses have not yet been observed and that the effects could be sustained by continued exposure.

In contrast to our data, Vaughan *et al.* (1984) reported that the exposure of male or female guinea pigs to 1 ppm O_3 continuously for 2 weeks caused increases in concentrations of serum Ch, TG, LDL, and VLDL but a decrease in feed intake (for a constant body weight). However, the differences in their results and ours should not be considered inconsistent or contradictory in view of reports for other O_3 effects that have been observed to display varied, and sometimes biphasic, patterns of response as functions of species, O_3 concentration, and exposure regimen (Werthamer *et al.*, 1974; Gardner *et al.*, 1974; Mustafa *et al.*, 1983).

In many human epidemiologic studies hypercholesterolemia, per se, has been correlated consistently with increased incidence of and mortality from coronary heart diseases (Gotto, 1979; Dawber, 1980); whereas, data supporting similar correlations with hypertriglyceridemia are less well substantiated (Gotto, 1979; Carlson, 1982; Kannel *et al.*, 1979). The magnitude of the changes we observed in normal circulating concentrations of rat serum Ch caused by 1 ppm O_3, i.e., approximately 2 to 3% in the concentration–response study and 5% in the sampling time study, is relatively small when compared to other effects of O_3 which have been reported. Given the present data and data from other studies, it appears, none-the-less desirable to explore further the effects which acute and chronic exposure to O_3 and other oxidizing air pollutants may have on lipid metabolism.

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